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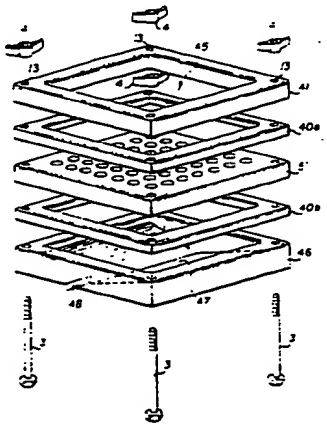

(57) Abstract

Method and apparatus for synthesizing a combinatorial library of families of biopolymers, such as polypeptides, oligonucleotides and oligosaccharides, on a reusable, spatially addressable solid phase plate (5'), typically in arrays of 4x4 to 400x400. In the case of peptides, such as synthesis of hexapeptides, the library contains one to three, typically two, positions in the sequence which are uniquely identified by the spatial address location. The preferred plate (5') embodiment employs a hydrophilic polar multi-functionalized polymer film coating discs or "winks" (50) of porous polyolefin which are removably received in holes (51) in the plate (5'). The plate (5') is employed with a vacuum block system (46, 47, 48) to assist in washing, deprotection of protected monomers, such as FMOC protected amino acids, and screening of immobilized, synthesized hexapeptides, for example, to determine which synthetic hexapeptides specifically bind to functional target proteins, such as enzymes, receptors and antibodies. Following identification of the known synthetic polypeptides giving the greatest affinity for the arrayed positions in the sequence, optimal binding for the complete peptide sequence is determined by an iterative process replacing formerly mixed positions with known amino acids at defined spatial addresses.

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SPECIFICATION
METHOD AND APPARATUS FOR PEPTIDE SYNTHESIS AND SCREENING

BACKGROUND OF THE INVENTION:

This invention relates to methods and apparatus for preparing a non-volatile, reusable, Addressable Synthetic Biopolymer Combinatorial Library (ASBCL) having known sequences at identifiable designated addresses arrayed on a permanent substrate, which library is rapidly creatable by a unique and simple slotted block system. The invention also relates to the use of ASBCLs to screen for sequences having biologic, biochemical, biomedical or therapeutic activity relative to a specified target. The invention permits rapid optimization of leads for identification of active components when applied to the specific area of peptides. The invention may be termed PILOT, for Peptide Identification and Lead Optimization Technique.

Moderate length peptides have attracted considerable research and commercial interest by virtue of the properties some exhibit in enhancing, blocking or otherwise affecting the activity of receptors, microbes, and other molecules deemed biologically significant. Specifically, hexapeptides have proven to have a sufficient chain length to block much larger molecules such as receptors, enzymes and antibodies. Thus, synthetic and natural hexapeptides have exhibited diverse therapeutic properties, among them: Antimicrobials with minimum inhibitory concentrations an order of magnitude less than known natural antimicrobial peptides; bactericides; antivirals; activity as antigenic determinants; and the like. The problem is that there are 64 million (64m) hexapeptide combinations for the twenty L-amino acids, and another 64m for the D-amino acids. Indeed if the selection were made from all of the L and D combinations the number amounts to 4.096 billion. Since there are in turn millions of biologically/medically

significant targets, preparing a complete suite of just 64m L-hexapeptides and assaying activity for each of the millions of targets is, practically speaking, an infinite, and therefore, impossible, task.

Accordingly, the Synthetic Peptide Combinational Library (SPCL) approach has recently resulted in a manageable approach to the problem of screening for a unique hexapeptide among the 64m that is the most active for a given target. In order to be feasible, libraries of large numbers of hexapeptides, on the order of 100,000 or so at a time, must be prepared in quantities sufficient to result in a positively determinable reaction.

There are currently five basic library techniques offered: viral approachs (originated by George Smith of LSU, and by Cetus and Affymax independently); the Chiron Geysen polyethylene pin system; the Houghten approach using Tea-bags; the Selectide bead approach; and the Affymax Chip approach. The latter four have distinct advantages over the viral approach in which peptide libraries are displayed by bacteriophages (viruses that prey on bacteria). A short degenerate oligonucleotide encoding all combinations of a short peptide sequence is cloned into Gene III or VIII of a filamentous phage and expressed on the phage surface. Recombinant phage are screened with the target molecule (e.g. receptor), and phage expressing a certain peptide that binds to the target are identified. Nucleotide sequence analysis of the recombinant Gene III or Gene XIII identifies the peptide sequence displayed by the binding phage.

The problem with the viral approach is that the range of peptides is limited to those tolerable by virus and E. Coli. That is, only a limited suite of peptides can be produced from among the 64m possible hexapeptides, and likewise for the even greater numbers of longer peptides. Additionally, only L- amino acids are allowed, and each individual hexapeptide of the library is produced within the phage as fusion products. This reduces the flexibility of the sequences, and may mask them entirely.

Methods for synthesis and display of peptides on

surfaces as well as techniques for binding from partial sequences were reviewed by H. Mario Geysen in Geysen, H.M. et al, Synthetic Peptides as Antigens, Wiley Chichester (Ciba Foundation 119), 130-149 (1986), shown in U.S. Patent 4,833,092 (19____). Geysen used functionalized polyethylene pins clustered to fit 96 hole microtiter plates. This Chiron system also relies on the method shown in Rutter-Santi patent 5,010,175 of preparing peptide sequences by providing constituent amino acids in concentrations relative to each other based on their relative coupling constants so that the resulting peptide mixture contains peptides in equimolar amounts. Chiron reports that its recent patent 5,194,392 entails synthesizing up to 1000 peptides a day on special pins, evidently a reference to the Geysen pin system of 4,833,092. The peptides can be used to "map" regions called epitopes in any protein of interest, such as antigen regions that trigger an immune response by T-cells.

The Selectide bead approach uses vast quantities of spherical crosslinked polymer beads (Millipore or Cambridge Research Laboratories polyacrylamide beads or Rapp Tentagel polystyrene) divided into 20 equal piles, each of which then has a different L-amino acid coupled to all the beads in the pile. The bead piles are then combined and thoroughly mixed. The resulting single pile is again divided into 20 different piles, each of which is reacted with a different one of the 20 different L-amino acids. This Divide, Couple and Recombine process (DCR) is repeated through six reactions to produce hexapeptides bound to the beads. The beads are then screened against a "target" molecule which is marked with a conjugated enzyme, such as horseradish peroxidase. The target "sticks" to active hexapeptide(s). The bead is rendered visible by adding a substrate for the enzyme which converts it to a colored dye which is precipitated within the beads, and then the visually identified bead(s) are picked out with tweezers. The peptides on the beads are then analyzed, for example by the Edman sequencing method,

and soluble versions produced in a synthesizer. The initial screening (locating the target bead(s)) takes only days, the makeup of each identified hexapeptide is unknown, and the analysis and synthesis for confirmation and further work takes much longer.

The Houghten (Iterex) Tea-Bag method, shown in U.S. Patent 4,631,211, employs methylbenzhydrylamine (MBHA) polystyrene beads in a number of foraminous containers, e.g. porous polypropylene bags (Tea-Bags), to prepare a truncated SPCL. In order to shorten the processing time, the Tea-Bag process employs partially known, partially undetermined hexapeptide sequences in repeated screenings, followed by iterative resynthesis to replace the unknown AA sequence positions with known AAs, i.e., A-$O_1O_2O_3$XXX, A-$O_1O_2O_3O_4$XX, etc. The method works on the assumption that a biologically significant response can be detected from a solution which contains hundreds of thousands of inactive components.

The Tea-Bag process typically uses 18 of the 20 L-Aas (cysteine and tryptophane are omitted in the initial library for ease of synthesis), starting with 104,976 combinations of non-determined tetrapeptide resins (XXXX-peptide resins) in 324 aliquots, and adds the 324 known dipeptide sequences (18^2) in the terminal two positions. For epitope determination of antibody binding, the 324 pools are screened to see which best inhibits binding of the target antibody with its natural antigen. The most active amino terminal dipeptide sequences are then incorporated into a further set of 20 pools in which the third residue is varied. These are rescreened for low IC. The most active sequences are again reincorporated iteratively to define positions 4-6 to finally obtain a characterized active hexapeptide.

The foraminous container of the Tea-Bag must retain the solid phase beads, yet have a sufficient number of openings to permit ready entrance and exit of solvent and solute molecules at the reaction temperature, but bar exit of the solid phase. While the synthesis is the standard Merrifield technique, new linking groups that attach the

X_n-peptide to the styrene bead supports are disclosed. This process can be characterized as not calling for a continuous support, and it is not addressable.

The Affymax "chip" approach described in PCT publication W090/10570, and in Fodor, P.A. et al, Science, 251 (1991) 767, is a method for multiple peptide synthesis on a solid support which uses synthesis and flourescent detection on the silica surfaces of flow through cells, photolabile protecting groups and photolithographic masking strategies to make arrays. Photolabilely-blocked amino groups are chemically attached (bonded) to a silicon chip, then irradiated through a patterned mask to selectively remove the blocking groups in a pre-arranged pattern. An amino acid will bond by addition only to the irradiation exposed areas. Additional masks are imposed and radiation applied as a prelude to adding second amino acids. Each amino acid added can include a blocking group so that further addition to that site occurs only after irradiation unblocking. Repeating the process with plural masks builds location specific polypeptides. When the chip is exposed to the target molecule, it may stick to one or more locations. By checking coordinates on a map of the chip, the peptide is identified. However, this process does not work with target molecules stuck to, or part of, cells, and there are exposure problems during processing, i.e., some AA's are light sensitive and cannot be used. Further, the reactions at the surface are not complete; for example, where reaction completion is only 90%, by the 6th iteration to obtain a hexapeptide, only half of them will be made properly.

There is clearly a need in the art for a peptide synthesis and screening process that is rapid and accurately identifies the active peptides from amongst those in an extended, reusable SPCL. Accordingly, it is among the objects of this invention to provide methods and apparatus for creating a non-volatile, reusable Addressable Synthetic Biopolymer Combinatorial Library (ASBCL) having known amino acid sequences at identifiable designated addresses arrayed on a permanent substrate for

rapid screening of target receptors and molecules by use with the Peptide Identification and Lead Optimization Technique (PILOT) which employs a simple slotted block system for rapid multiple amino acid addition reactions to build peptides of known sequences at identifiable designated addresses in an X-Y coordinate array on a variety of planar substrates, and preferably uses sintered polyolefin discs having thereon ultra-thin HPMP films, called thin-film HPMP Winks, in plate, slotted block and vacuum block assemblies, to permit display of assembled synthon molecules in an unhindered, near-aqueous environment, and thereby permit high quality peptide ligand synthesis, high ligand loading, efficient binding of radioactive target molecules and facile removal of unbound targets by suction washing, and repeated regeneration thereof.

SUMMARY OF THE INVENTION:

The invention comprises methods and apparatus for preparing non-volatile, reusable, Addressable SBCLs (ASBCLs) or SPCLs (ASPCLs), having known arrayed dipeptide amino acid sequences incorporated at any desired and known position within any biopolymer (e.g., polypeptide) sequence of length producible by chemical synthesis methodology, in which up to 6 positions (typically 4) may be composed of mixtures of residues, the remaining positions comprising specified amino acids. The arrayed peptides are identifiable from designated addresses provided on a permanent, reusable substrate-containing plate which permits creation of an ASPCL, typically within a day. While the discussion herein is with reference to hexapeptides by way of example, the principles of the invention are applicable to any binding determinant biopolymer to produce an ASBCL for interaction with any biologically significant target. These library peptides are also termed ligands.

The ASBCLs of this invention can uniquely identify the binding determinant biopolymer, e.g. an active hexapeptide, at a unique X-Y coordinate axis upon bonding

with or adhering to a fluorescently labeled, radio-labeled or enzyme-linked target molecule or receptor, e.g., in solution flowed into contact with the ASPCL-bearing substrate. The amino acids and peptide sequences are substantially equimolar concentrations on the substrate, so good quantitative activity is determinable by the fluorescent or radio intensity, or by the optical density of the dye product formed in the enzyme linked probing.

In addition, the hexapeptide array is permanently bound to the substrate i.e., is non-volatile, and at each substrate site on the array plate on the order of 1μ mole or more can be bonded. The target molecule can be washed off the substrate plate so that it can be reused repeatedly, particularly for diagnostic testing, as well as rapid active peptide screening on a wide variety of target receptors. For example, a preselected library of peptides, or any other condensation chemistry-based screening agent, may be permanently bonded to a substrate as a diagnostic tool. One example involves exposing an ASPCL plate of this invention to one or more aliquots of a serum which requires diagnosis, and then visualizing binding by passing a flourescently or radiolabeled anti-IGG antibody over the ASPCL plate. Consequently, one or more conditions, such as the presence of antibodies to HIV-1, or the presence of other viral infections can be rapidly and simply diagnosed.

More broadly considered, the peptide may be any biopolymer. Thus the term ASBCL applies to the library on the identifiable designated addresses arrayed on the permanent, reusable substrate plate. It should be understood that the term "substrate" as used herein includes broadly but is not limited to: a) polyolefin plate alone, or, preferably, an activated plate carrying a plurality of substrate discs; b) a plate with a bead or gel substrate, amino functionalized or bare, (receptor substrate); c) such beads or gels with spacer arms, amino-functionalized or bare, (spacer receptor substrates); and d) reacted substrates i.e. such substrates above with one or more Aas or peptides linked thereto.

The apparatus system of this invention comprises employing an inert substrate support plate, such as a polyolefin polymer, having a plurality of discrete sites (such as holes for retainingly engaging removable substrate discs, called winks, or small, well-like, shallow flat-bottomed circular or square depressions) in a spaced array, e.g., 4x4, 10x10, 16x16, 20x20, 40x40, 100X100, 400x400, or any other desired number. The wink-holding plate array is presently preferred. Each of the sites are permanent uniquely addressable locations for assembly of the biopolyer chains or attachment of preactivated molecules. Each site includes an amino functionalized substrate media such as a sintered polyolefin (polyethylene or polypropylene) disc, glue-bonded beads, or chemically grafted polymeric films, which may be gel-type films. Any conventional peptide substrate, media, or addition chemistry-based agent substrate, may be used.

The preferred substrate is a 1/4" diameter sintered polyethylene disc of approximately 1/8" thickness, which is coated with an ultra-thin Hydrophilic Polar Multi-functionalized Polymer (HPMP) film, herein called a "wink". The film and methods of anchoring to the polyolefin are disclosed in more detail in our co-pending priority application U.S. Serial No. 08/019,725, the disclosure of which is incorporated by reference herein to the extent needed.

The film is preferably carboxymethyl dextran carbodi-imide coupled to the polyolefin disc surfaces after functionalizing them with a diamino-substituted polyethylene glycol spacer arm.

Another example of a film is a hydrolyzed or partly hydrolyzed chitin (herein chitin/chitosan) having from about 1 to about 100 sugar moieties per amino group which may be coupled to the polyolefin disc surface with mono-amino substituted polyolefin glycol spacer arms after functionalizing most of the amino groups with protective functionalities such as t-Boc or Fmoc that can be removed later. The degree of alkaline hydrolysis of the chitin

controls the solubility of the resulting chitosan. The amino group and hydroxyl on the sugar moieties permits the chitin/chitosan to be bifunctional providing a wide variety of tether linkages to ligands through either or both types of groups. The amino groups provide a controllable positive charge that may be advantageous in certain affinity binding environments. The polymer film molecular weight may range from about 50,000 to about 1 million.

The films permit display of assembled synthon molecules (ligands) in an unhindered, near aqueous environment. These substrates permit high quality peptide (ligand) synthesis, high ligand loading in the film in the range of 50-100 nmole loading, efficient affinity binding of radioactive or flourescent labeled target molecules, and easy removal of unbound targets by suction washing, and repeated regeneration and reuse of the library. The substrate discs are easily pressed into and removed from holes bored partway through a substrate carrier plate, which preferably includes at least one smaller through-hole to permit rapid and thorough suction removal and washing of the array of substrate discs in the carrier plates. A vacuum base plate is used with the array carrier plate to facilitate the excess target solution removal and washing steps in the common synthesis and probing steps.

Examples of bead type substrates are polydimethylacrylamide (PDMA) particles, silica beads, MBHA polystyrene beads, and the like, which are glued to the substrate areas of the support plate. The presently preferred bead substrate is Kieselguhr-encapsulated PDMA particles (Pepsyn-K from Millipore Corp.), secured to a polyethylene plate with a low temperature (<100 degrees C) hot-melt polyethylene adhesive. The preferred polymeric film is chemically grafted to the surface of the wells by a process disclosed herein, and is particularly useful for screening involving large proteins.

Two methods of attachment of amino-functionalized polymers to form substrate areas on the plates are disclosed by way of examples of the principles of the

invention; in situ polymerization (disclosed in detailed examples); and bonding of a pre-polymerized material to activated areas on the plate (disclosed in general). The first polymerizes acryloylated monomers and crosslinking agents onto acryloyl groups attached to the areas of the polyolefin surface (plate activation). This establishes a gel-type polymer covalently grafted into the depressions. This polymer, since it may possess low structural strength, i.e. it need not have high structural strength, can be prepared from monomers at low concentration and with a low molar percentage of cross-linking. The resulting gel substrate materials are therefore highly permeable to proteins, thus greatly improving the sensitivity of detection. The gel film may then be amino functionalized.

A spacer arm derivative is attached to the functional amino groups of the resultant films. These spacer arms, which are also used for the glued beads, increase sensitivity since they reduce unfavorable steric and electronic interactions between the incoming tagged protein and the polymer backbone. An ideal spacer is non-hydrophobic, incapable of forming aggregates by hydrogen bonding, and typically longer than 10Å. A variety of materials, including functionalized polyethylene glycols, sugars, and short natural and unnatural peptides may be used as spacers.

The second (pre-polymerized) method of attachment of functional polymers involves attachment of preformed amino-functionalized polymers to the designated areas on the inert (polyolefin) support plate itself, or on the sintered polyolefin winks (discs) which are retained in the plate. The preformed polymer itself serves as a spacer arm, and access of proteins is improved as compared to the in situ gel type polymers. Examples of preformed amino-functionalized polymers include polyethyleneimine, polyallylamine, long chain functionalized sugars (e.g. dextrans and chitosans), polyamino acids (e.g. poly-L-lysine) and the like. They can be coupled to acid chloride activated plate areas by reactions of the type

described herein. We prefer to use a 500,000 MW dextran that is amino-functionalized pre- or post-attachment to the plate area, or to the winks.

For the array preparation steps, an elastomeric sealing gasket having a plurality of holes or slots therethrough aligned with the substrate areas is placed over the substrate array plate or wink carrier plate, and a slotted block is placed over the gasketed plate so that individual slots align with rows of substrate areas. When the wink carrier plate is used, a solid non-apertured gasket is placed below the wink plate to seal the through-holes, and then placed on a base plate. The assembly of the plate, slotted block and appropriate gasket(s) is clamped together for use.

Since each area is part of a predetermined array, each defined substrate area has a unique X-Y coordinate address, such as: Row 1, position 1; Row 2, position 20; Row 3, position 78; to Row X_n, position Y_m. Any desired address system may be used, such as sequential numbers for each succeeding area, dual alpha system (AA, AB, AC etc.), or alpha numeric (A1, A2...B1, B2...).

The slotted block has a height sufficient to provide a well of sufficient volume to receive reaction solution having selected moieties for bonding with the exposed substrate address area, or for reaction with a previous moiety. Each well can receive a different reactant, e.g. a blocked AA, so that each row has a different A_n position AA. Next, the reactants are removed from the slots in the block, e.g. by decanting or suction, then the amino blocking group removed by a deblocking agent, e.g. piperidine. Then the slotted block (and slotted gasket if such is used) is rotated horizontally 90°, and each slot well receives another, same or different, reactant so that the A_{n+1} position has a predetermined AA. Where the sequence of Aas are the same in each well at each block orientation, turning the block 90° produces all 400 combinations of dipeptides for a 20 slot block/400 substrate area plate system. Iterative application of 3 such plates, two positions being optimized at a time,

allows for the identification of the single optimal binding peptide from a 64m hexapeptide SPCL. The substrate areas can be quite small to provide peptides in adequate (picomolar) amounts, e.g., the 1/4" diameter winks.

As an illustration of another method of use of the system of this invention, a random sequence of all XXXX-tetrapeptides on Pepsyn-K beads is prepared, and these beads adhered to the substrate areas. Alternately and preferably, a polymeric film gel (HPMP) may be prepared on a plurality of winks, which are then reacted in a flask (100 ml/100 winks) of an automated peptide synthesizer to produce the random XXXX-tetrapeptide mix. These winks are then pressed into the holes in a wink carrier plate. The wink plate is placed on a border or periphery gasket on a vacuum block, and then overlain with another border gasket and a "window frame" border block and clamped.

Then, using the system of this invention, the terminal two Aas can be added thereto in the all-combinations 20x20 array via the rotation of the slotted block assembly. Alternately, a known dipeptide can be added to the end terminal by two cycles of reaction with the vacuum block, say all VY, i.e., VY at all positions. A reaction cycle is defined as deblocking the prior reaction step AA, and reacting with the next blocked AA. The resulting hexapeptides are screened (probed) by exposure to labeled targets. This is done in the vacuum block assembly.

A deductive process involving iterative resynthesis of successively smaller libraries can be used to successively characterize the resulting screening-active hexapeptide. Alternately, the procedure and apparatus of this invention can work from a defined middle dipeptide with random ends, followed by replacement of each end in sequence with known dipeptides. Likewise 4 or 5 residues may be mixed, or an array of any kind of peptide, including those including one or more non-natural Aas, can be employed on the reusable substrate of this invention.

The use of winks receivingly engaged in the support

address area is preferred for diagnostic or drug use applications, as single preselected, known-sequence peptide-containing winks can be prepared in place in the carrier plate, or separately prepared in an automated synthesizer and inserted in the holes in the support in specified address(es). Likewise, DNA moieties can be bonded to the support, in which case a 16x16 array, or an array of 16-4x4 subarrays, on a single plate is preferred.

Advantages of the apparatus and methods of this invention include: Synthesis of defined peptides, portions of which optionally can consist of redundant known or unknown (uncharacterized or non-defined sequence) mixtures which are bonded in micromolar amounts in defined arrays with known addresses so that a physical barrier (e.g. an appropriately apertured member) can permit simultaneous screening. Another feature is fluorescent or radio-labeled detection of binding, which provides higher sensitivity and is far more suitable for detection of low affinity interactions than the current Selectide or Iterex technology. The solid array support also permits inference of optional binding elements (e.g. AA sequences) from the spatial position (unique address) rather than requiring chemical determination of sequence.

The ASBCLs and ASPCLs constructed by the method and system of this invention are selectively variable at any two or more positions, while redundant (random selection of all combination) at several other positions (say, 3-6) within peptides or biopolymers of a wide range of size and structure. The system is also useful for screening (probing) by itself, or in conjunction with current methods (such as the Iterex Tea-Bag or Selectide methods), for any two or more AA position sequencing, and can be used for progressive refinement of initially identified hits (indications of activity). Because of the effectiveness of the support system of this invention, the separate zones (one or more support address area(s)) can be functionalized for synthesis of peptides at loadings as low as about .001 micromoles per cm^2, usually in the range of from about .05 to about 50μ mole/area, and 50-100 nmole

loading for HPMP winks.

The system also permits simultaneous or sequential synthesis by standard Fmoc or t-Boc-chemistry of identified areas (addresses) of distinct known or non-defined peptides, by attaching the slotted block to the substrate plate for simultaneously performing individual separate couplings in the slot compartments. By transforming the block orientation, arrays of peptides may be synthesized at any two positions within a peptide or biopolymer of any length. The previous or following Aas in the peptide sequence may be uniform across the entire substrate surface, and may be unique or consist of mixtures of one or more peptides of known or uncharacterized composition. Common steps can be carried out in the vacuum block system, and winks loaded with random peptides may be prepared in an automated peptide synthesizer.

While the same block is shown used in different orientations, e.g. rotating a slotted block 90°, two dissimilar blocks may be used in the array generating steps, such as a radial slot block (slots extending radially outwardly from a common center) in combination with a block with concentric circular (annular) slots, and the resultant array may be addressed by polar coordinates.

The system of the invention permits displacing the label on the target with a natural ligand to insure specificity of the identification. It also permits reuse of the substrate for repeated probing of the surface by alternative proteins i.e. exposure to different targets followed by washing. DMF washing is particularly easy by use of the vacuum block where the wash is removed by aspiration through the vacuum base. Different areas (addresses) may employ the same or different binding materials, e.g. Pepsyn K particles in one area, winks in another, and grafted films in another.

Although the method and apparatus shown herein are directed to definition of optimal binding linear hexapeptides, it has great applicability in different formats. Especially where the protein of interest, e.g.

cytokine receptor, binds a large ligand, then it is advantageous to insert the library within longer sequences, particularly those which are known to form stable secondary structures, as in loops, beta conformations or alpha helices. In the latter, since the library is then displayed on a cylindrical surface, it is of interest to construct the components 3 or 4 residues apart, the components being separated by helix-forming residues, such as alanine. For libraries based on loop structures, either end may be designated as a Cys residue which can then be coupled together by intramolecual disulfide bridges. Cyclic peptides, especially cyclic hexapeptides and cyclic decapeptides can be constructed on PILOT substrate matrices of this invention, and are especially useful for the relative rigidity of these molecules compared to their linear counterparts. Also, bogus pseudo array checking can be easily done with the system of this invention.

The novel PILOT ASBCL's and methods of this invention provide distinct advantages over the numerous alternatives discussed above in the Background to meet the need for developing new pharmaceutically useful compounds. The specificity of the binding may be uniquely established by side-by-side comparative processing of dual plates which are then probed, one with the presence of the natural ligand, the other without, and the two compared.

One particular advantage of the invention is that it allows detection by numerous methods, but it is unique in being suitable for detection with radiolabelled derivatives, with autoradiographic and counting methods providing the enhanced sensitivity vital for the detection of relatively low-affinity binding peptides which are present in picomolar amounts within pools containing thousands of other non-binding sequences. Use of ^{125}I labeling with Bolton-Hunter reagent provides sensitive and simple detection by auto radiography. With ^{35}S and ^{14}C labeling, arrays are recoverable and can be reprobed numerous times after scintillation counting of individual winks pushed out of the carrier (holding) plate.

Another advantage of the invention is that it allows for the use of standard substrate materials (e.g. HPMP winks or Pepsyn K for peptide synthesis, and HPMP winks or controlled pore glass for DNA/RNA synthesis), for synthesis on the plates, or for preassembly by automated synthesizers followed by arraying these for diagnostic applications. In library applications, the unique method of grafting in-situ generated polymers or attaching preformed polymers to functionalized polyolefin surfaces such as winks provides materials far better suited for screening methods than conventional particulate solids. A special virtue is the optical clarity of the HPMP film substrates of this invention, combined with their low intrinsic fluorescence which greatly enhances the sensitivity when used with fluorescent tags. Of even greater importance is that the substrate HPMP films of this invention are formulated to provide excellent penetration of proteins within their bounds, and side-by-side comparisons with prior art methods have shown significantly enhanced sensitivity with use of this invention.

This invention is unique in being suitable for construction of libraries containing monomer units of almost any kind, for example, bound together by ether, thioether, ester, amine, phosphate, amide or any such bond establishable by organic chemistry methods. Identification is performed solely through spatial recognition, and does not require sequencing, which is generally impossible with other than natural peptide and DNA units.

The PILOT ASBCL's and methods of this invention, are therefore unique, simple, generally applicable and readily duplicated. They provide high sensitivity detection by a variety of tagging procedures.

It is important to the application of the PILOT system of this invention as a general library method to equally incorporate amino acids from mixtures of Fmoc-amino acids. We have found that differences in incorporation diminished with increasing concentration,

and D-derivatives coupled at rates essentially the same as their L-counterparts.

However, rather than use the Rutter-Santi method of employing concentrations based on the coupling constants of the amino acids, we use a non-theoretical, empirical method of determining the molar ratios for equal incorporation of Fmoc-Amino acids from mixtures. These mixtures are used to prepare the random X_n-peptides. We employ 16 AA's to form a standard mixture, Ω. As used herein, Mix • Mix, $(Mix)_2$ or Ω-2 refers to two reaction cycles with the Ω mixture.

Based on Example 1 below, the molar ratios described in Table 1 below give substantially equal incorporation on HPMP winks as confirmed by both amino acid analysis and sequencing. Sixteen amino acids are incorporated in our standard mixture Ω. Mixtures may be incorporated at 6 or more positions in a core sequence, and "arrayed" at any other two or more positions. Bogus arrays may be constructed using conventional peptide synthesizers. Study of non-arrayed mixed winks in the presence and absence of natural ligand(s) shows whether a detailed array study will be able to uncover binding sequences. The molar ratios of Table 1 are obtained by coupling, AA's selected with 1.25 equivalents HOBt + 1.5 equivalents PyBOP and 1.5 equivalents 0.3M NMM in DMF after 10 minutes preactivation.

Table I

Molar Ratios For Equal Incorporation of Fmoc-Amino Acids From Mixtures

Fmoc-Derivative	Molar Ratio	Fmoc-Derivative	Molar Ratio	Fmoc-Derivative	Molar Ratio
L-Nle*	1.00	L-Ala*	0.79	D-Nap*	1.50
L-Leu	1.00	L-Ser(tBu)*	1.50	L-Tyr(Tbu)*	1.70
L-Val*	1.60	L-His(Trt)*	2.10	L-Phe*	1.00
Gly*	0.60	L-Gln(Trt)*	2.20	L-Asp(OtBu)*	1.40
D-Ala*	0.79	L-Pro*	1.15	L-Glu(OtBu)*	1.20
L-Lys(tBoc)*	1.36	L-Arg(Pmc)*	3.00	L-Thr(Tbu)	2.00
L-Asn(Trt)	2.45				

"Nap" is 3-(2-naphthyl)-alanine; the 1-naphthyl derivative couples similarly. The AAs marked with * are used in the Ω mixture.

DRAWINGS:

Fig. 1 is an isometric view of an assembled PILOT slotted block system apparatus, partly broken away to show the various parts in proper alignment and ready for introduction of AA's for reaction with the substrate areas or moieties thereon;

Figs. 2a and 2b are section views taken in elevation along line 2-2 of **Fig. 1** showing two alternative structures of a substrate area in detail;

Fig. 3 illustrates using a combination of two different blocks, one radial and one with concentric annular slots, with a circular support plate to produce a circular array;

Fig. 4 is a section view of the concentric annular block taken along line 4-4 of **Fig. 3**;

Fig. 5 is an isometric view of the peripheral frame system of this invention for functionalizing plates prior to condensing biopolymers thereon;

Fig. 6 shows in exploded isometric view the presently preferred embodiment of the slotted block assembly of **Fig. 1** employing a vacuum disc-holding array plate for the array preparation steps;

Fig. 7 shows in exploded isometric view the use of the vacuum plate on the vacuum base for common peptide synthesis and probing steps;

Fig. 8A is an enlarged vertical section view through one hole of the vacuum block with a single vacuum draw and drain hole below the sintered disc "wink" in place in an array hole;

Fig. 8B is a vertical section-view through an alternate embodiment of the vacuum plate with multiple vacuum/drain holes; **Fig. 9** shows in isometric a slotted gasket used above the plates of **Figs. 1, 6 and 7** in place of the multi-hole array gasket 10 of **Figs 1 and 5**;

Fig. 10 is a graph of the percent amino acid vs. molar percent incorporation in equi-molar mixtures to prepare the Ω mixtures used herein; and

Fig. 11 shows the results of the ^{35}S streptavidin

array test.

DETAILED DESCRIPTION OF THE BEST MODE OF THE INVENTION:

The following detailed description illustrates the invention by way of example, not by way of limitation of the principles of the invention. This description will clearly enable one skilled in the art to make and use the invention, and describes several embodiments, adaptations, variations, alternatives and uses of the invention, including what we presently believe is the best mode of carrying out the invention.

Referring to **Fig. 1** the slotted block system of this invention 1 includes a square base 2 which has or receives orienting members, such as a plurality of guide and securing rods 3 with wing nuts 4. The square base 2 receives a square substrate support plate 5 which includes an array of areas 6. There are three basic variations in the substrates: in situ beads, film, or discs as shown in **Figs. 2a, 2b** and **8a/8b**, repectively.

In a first, embodiment shown in **Fig. 2a**, each area 6 includes substrate 7 (in this case beads) secured in depression 8 by a suitable glue 9. The second embodiment of **Fig. 2b** shows a grafted polymeric film as the substrate 7 in depression 8. **Figs. 8a** and **8b** show the porous sintered polyolefin HPMP coated disc, which is the presently preferred embodiment.

Overlying the substrate support plate 5 is a square gasket 10, preferably a sheet of chemically inert elastomeric material (e.g., Viton or silicon rubber), having an array of holes 11 therethrough which are the same size as and in alignment with the substrate areas 6. See **Figs. 1, 2a, 2b, 3, 6** and **9**. The gasket functions to prevent leakage between individual substrate areas 6 or discs 50. An alternative slotted gasket 10a, shown in **Fig. 9** may be used in place of multi-holed gasket 10, but it must be rotated with the slotted block 12. The slotted gasket 10a may be glued to the underside of the slotted block 12. Still another alternative is to provide O-rings, one per array area, in a groove concentrically

surrounding each area. Or a groove can be provided on the underside of each slotted block surrounding each slot, which groove receives a round (in cross-section) seal strip.

A square slotted block 12, having holes 13 therethrough to receive the guide/securing rods 3 is placed over the gasket 10. This block 12 includes a plurality of slots 14, 15, 16 etc. therein, which are aligned with and extend a full row width of the substrate areas 6. The slots may be identified, as by the alpha designations A,B,C etc. shown. Note in the substrate support plate 5, additional corresponding rows L,M,N... are shown. In a typical block 12, there will be some 4 to 10 and as many as 400 such slots. Fastening the wingnuts 4 secures the assembly together in the proper orientation and prevents leakage between adjacent slots and substrate areas when individual reaction solutions are placed in the wells formed by the slots. **Fig. 6**, the presently preferred embodiment in which the wink carrier plate 5' is used, employs the same top gasket 10, but uses a hole-less bottom gasket 49 to seal between adjacent holes.

Continuing with **Fig. 1**, after reaction, removal of solution, washing and deprotecting, the square block 12 is rotated by 90° and selected solutions are introduced in the chosen slots, to produce a known array of dipeptide sequences. This cycle can be carried out with the apparatus of **Fig. 6** as well.

Fig. 3 shows an important variation involving a round substrate support plate 20 having a concentric/radial array 21 of discrete substrate areas or holes 6. A gasket 22 also includes a corresponding concentric/radial array of holes 23. In this embodiment, two slotted plates may be employed sequentially: slotted radial plate 24, and concentric slotted plate 25, in either sequence, 24, 25 or 25, 24. There may be fewer radial slots 26 than the radial array of holes 23 or substrate areas 21, in which case the block 24 may be turned between application of reactants (e.g., AA solutions). The inner concentric segments 27, 28, 29 etc. may be secured in spaced

relationship by horizontal rods 30, 31, which are spaced above the bottom 32 of block 25 to insure access of solution to all relevant substrate areas. In **Fig. 2** the base plate and pins are omitted for clarity.

Fig. 4 shows in vertical cross section the construction of the concentric slotted plate 25 in which outer ring 27 is spaced from inner core 34 by rod 31. A series of tubular spacers 35, 36, 37, 38 on rod 31 space the concentric intermediate rings 28, 29 from core 34 to provide concentric annular slots. The rod 31 may be countersunk in bore 39.

Figs. 5 and **7** show a border frame assembly for functionalizing the substrate areas 8 on support plate 5, e.g., with common Aas, or mixtures of Aas, and for deblocking, washing, probing (screening) and addition of spacer arm derivatives. An edge gasket 40 is placed on the substrate plate 5, then a border frame 41 is placed thereover and secured with clamps 42, 43. This provides a central well 45 for the entire array for simultaneously receiving the appropriate solutions for the functionalizing chemistry.

Fig. 7 shows a border frame assembly in association with a vacuum base 46. In this embodiment the wink carrier plate 5′ is sandwiched between two identical edge or periphery gaskets 40a, 40b and placed on vacuum base 46 having a trough-shaped interior cavity 47 and an aspiration outlet tube 48. The border frame 41 is placed on top of gasket 40a and the entire assembly clamped together, by rods 3 through holes 13 which are secured by wingnuts 4. This is the preferred assembly for common steps, e.g., of adding the previously determined or selected di, tetra-, hexa-, etc, peptides and for deprotecting, washing and probing. The tube 48 is connected to a vacuum source, such as a water aspirator, which sucks excess or spent solution through the porous granular sintered winks (see **Figs. 8a** and **8b**) and out trough 47.

Figs. 8a and **8b** are enlarged vertical section views of the porous HPMP winks 50 which are easily insertable in

holes 51 bored partway through carrier plate 5'. A slight tapered shoulder 52 permits drainage via hole 53 when suction is applied from below. The wink diameter is typically 1/4", and hole 53 is 1/8". Pressure P from finger 54 is sufficient to press-fit the winks 50 into holes 5'. A dowel or Q-tip 55 inserted in hole 53 is sufficient to remove the wink. **Fig. 8b** shows a variation in which multiple drain holes 56, 57, 58 etc. may be employed. A smaller dowel or comb-like pusher may be used to remove the winks by insertion through holes 56-58.

The wink carrier plate 5' securely holds porous polyolefin discs 50 throughout the course of the array and probing. Dextrainized winks are prepared as described above. Common unarrayed sequences are assembled on the winks using commercial synthesizers. They are then mounted in plate 5' as shown in **Fig. 8a**. To directly perform an array the plate is mounted in the slotted block apparatus of **Fig. 6** with a solid viton gasket 49 between it and the base plate, and a regular holed gasket 10 placed between the plate 5'and the slotted block 12. Coupling of one dimension of the array is then performed. The plate is then transferred to and secured in the vacuum base apparatus of **Fig. 7**, along with edge gaskets 40a and 40b, and the window frame block 41, and washed with DMF (e.g., shaken with DMF for 30 seconds) then the wash is removed by aspiration through the base 46. This washing is extremely efficient in comparison to the bath technique of **Fig. 1**. This is followed by Fmoc removal steps (deprotection), and more washings performed analogously. The second dimension of the array may then be performed, or common sequences introduced, as required. Following assembly of the array, the completed plate mounted in the vacuum block of **Fig. 7** is treated with TFA + scavengers to remove side-chain protection. Following washing with methanol, DMF and water, the plate is then washed and thoroughly equilibrated with assay buffer. The radio-labelled protein is then introduced, and the plate probed as appropriate (10 minutes to 2 hours). A parallel plate produced in a second apparatus of **Fig. 7** can be used to

simultaneously determine the specificity of binding (i.e., by addition of natural ligand). After incubation, the plate 5′ is suction washed rapidly with 4 additions of buffer (20 Ml), removing unbound label. Binding may be determined by autoradiography. With weaker isotopes, the individual winks may be punched into scintillation vials and counted. This is a simple and quantitative procedure. Of great importance, we have found that, in a variety of cases, the winks can be recovered, the radioactivity displaced, and the winks reused for similar or different assay purposes. The reusability of the system is one of its key attributes.

The following example protocols describe the sequential steps of the method:

Example 1 - Determination of AA Mixture Proportions for Equimolar Incorporation on Substrates - Empirical Method:

The component amino acid (Fmoc-X-OH,1 equivalent) is mixed with Fmoc-Nle-OH (1 equivalent), then dissolved and activated by the addition of PyBOP, HOBt and NMM solutions. After 10 minutes the mixture is added to Nva-PAL-Pepsyn K support (5 mg). After 2 hours the support is washed with DMF repeatedly, treated with 30% piperidine in DMF (to remove incorporated Fmoc- groups), washed with DMF, and methanol, then treated with TFA/water (95:5) for 2 hours. The TFA solution is expelled into a vial, a known proportion of it dried down in vacuo, and the resultant mixed dipeptides X-Nva and Nle-Nva are hydrolysed with 6M Hcl at 150 degrees for 1 hour. The relative incorporations of X and Nva are then determined by amino acid analysis.

A graph is constructed plotting molar % X (in this initial round X is 50%) against molar percentage incorporated; and the curve which results when using extremities points at 0 and 100% is used to predict what molar percent X would give equal incorporation to that of Nle. The molar percent incorporation of an individual amino acid is the amount of the amino acid divided by the sum of the amino acid + Norleucine + Norvaline (i.e.,

AA/(AA + NLE + NOR) = % incorporation). As shown in Fig. 10 the molar percent incorporation is then plotted against the percent amino acid in the mixture. The zero and 100% data points are also included. Draw a line from the 50 percent point on the y-axis to the curve and then drop a line from that point to the x-axis and determine the percent molar incorporation necessary to obtain a 50 percent amino acid mix. In this example, the first evaluation was made with 50% Asparagine and 50% Norleucine. This gave a mixture of approximately 30% Asparagine and 70% Norleucine. A refined evaluation was made with 67% Asparagine which gave a mix of 45% Asparagine/55% Norleucine. The final value was determined to be 71% Aspargine to achieve a 50% mix.

The process is repeated using the predicted molar percent X to confirm and, if necessary, iteratively refine the molar percent. This method has been applied to all 20 natural L-amino acids, most D-amino acids, and several unnatural amino acids, such as beta-alanine and 2-napthylalanine.

For any desired library mixture, the amino acids are selected, mixed in the correct ratios, activated, coupled to the support, and the equal incorporation confirmed by analysis. For the 10 amino acid library the subject of these examples the following recipe gives equal incorporation: Fmoc-L-Nle-OH 0.188g; Fmoc-L-His(Trt)-OH 0.73g; Fmoc-L-Pro-OH 0.24g; Fmoc-L-Gln(Trt)-OH 0.797g; Fmoc-L-Tyr(Tbu)-OH 0.398g; Fmoc-Gly-OH 0.093g; Fmoc-L-Phe-OH 0.206g; Fmoc-L-Arg(Pmc)-OH 1.25g; Fmoc-L-Glu(OtBu)-OH 0.288g; and Fmoc-D-Ala-OH 0.130g. To these mixed amino acids were added HOBt 1.614g, and the entire solids totally dissolved in DMF and made up to a volume of 40 Ml. For coupling, 10 Ml of this solution, called MIX solution, is added to 1.71g PyBOP reagent, mixed, 0.35 Ml of N-methylmorpholine is added, remixed and left for 10 minutes. This solution is adequate to completely cover and react a single 10 x 10 plate giving equal incorporation. Table I and its related description above show the quantitative amounts in this example converted to molar

ratios.

Example 2 - Preparation of A "Beaded" Plate (Support Plate With Array of Bead-Type Substrate Areas):

The sequence BAla-Bala-Bala-Nle-Bala-Nle-Bala-Bala was assembled on 0.2 mmol/g Pepsyn-K (Millipore) functionalized by treatment with ethylenediamine using a Milligen/Biosearch model 9600 peptide synthesizer using standard BOP + HOBt coupling protocols. Ace Hardware Hot Melt adhesive was cut into thin sections and melted at as low a temperature as possible on a flat PTFE sheet to produce a thin sheet (in a range of from about .2 to about 1.0mm thick) of hot melt adhesive (HMA sheet). The PTFE sheet was removed from the heat, and dry, peptide-bearing Pepsyn-K beads were sprinkled over the melted glue surface and gently patted down. After several hours of cooling, excess beads were removed and the glue sheet lifted off the PTFE sheet, then punched into appropriate circles (e.g. 1-10mm dia) with a standard hole punch. The resultant discs were then attached to an array of shallow, dished wells in a polyethylene sheet using a Black and Decker Thermogrip glue gun adding a dab of glue in the well and pushing the discs down firmly. The discs can be reinforced with polypropylene or metal mesh. Typically, each patch bears 5 mg of beads, having 1 micromole of spacer arm linked Pepsyn-K. The top right hand corner of every plate is notched or drilled as a reference to prevent the plate being incorrectly aligned at any step.

Example 3 - A Preparation of A Polymeric "Film" Plate:

A 10mm thick plate of linear high density polyethylene is floated in a water bath at 70°C and treated with 5M chromium trioxide in 5.3M sulfuric acid for two hours. The plate is washed with water many times, then with methanol, and then with ether, and dried under vacuum. The surface bound carboxylic acids are converted to acid chlorides by treatment with 20% thionyl chloride in chloroform for two hours. The plate is rapidly washed with chloroform, then ether, and dried under a stream of

nitrogen and used immediately. This acid chloride functionalized plate can be derivatized by a variety of reagents to introduce many functionalities. Linear polymers, such as polyethylenimine, poly(amino)functionalized polyethylene glycols, and saccharide may be added to the plates by conventional chemistries. For example, for introduction of acryloyl groups, the plate may be treated with either a solution of N-(3-aminopropyl) methacrylamide hydrochloride and triethylamine in DMF, or it may be treated with neat (undiluted) diaminopropane for two hours, followed by washing thoroughly with water, then methanol, then ether, and then treated with a solution of acryloyl chloride and disopropylethyl amine in THF. The plate is washed well consecutively with methanol and ether, and dried under vacuum. The plate is now ready for grafting or casting of a gel film thereon as the substrate in the specific address areas.

The gels which may be cast into the wells of the substrate plate or grafted to the acryloyl groups on the surface of a film plate can have a variety of concentrations, cross-linking levels, functional linkers and amino linker loading. To prepare a typical gel, under nitrogen, a 5 ml portion of deoxygenated water (under vacuum for 20 minutes) is added to 18.5 mg of bisacryloyldiaminohexane, 295 mg of dimethylacrylamide, and 186 mg of the monoacrylamide of 1,6 diaminohexane hydrochloride. This is filtered onto 15 mg of ammonium persulfate and treated with 30 uL of Ph 6 TEMED solution in water. In a glove bag under nitrogen, the monomer solution is rapidly transferred to each well of the plate. The plate is sealed in a plastic bag with an open beaker of deoxygenated water and allowed to gel. After curing over night the plate is washed with water and soaked in 1N sodium hydroxide for 2 hours. Two water washes of 15 minutes each followed by at least four washes in DMF give a plate which is ready for peptide synthesis.

Example 4 - Spacer Arm Derivatization of The Film:

Optionally a gel film plate of the type in Example 3 may have a tetrapeptide spacer attached to the substrate. A plate prepared as in Example 3 had Fmoc-beta-alanine (Bala) coupled to it (standard PyBOP + HOBt/NMM procedure, 2 hours). Following thorough DMF washes, the plate was treated with 30% piperidine in DMF (1, 45 minutes). The plate was washed 2 times with DMF, and the piperidine treatments and subsequent washes were pooled and read spectrophotometrically at 301 nm to determine the Fmoc-loading (in this example 2 micromoles per substrate area). Three more coupling cycles were then performed adding Fmoc-epsilon-aminocaproic acid twice, then beta-alanine again to give the final Bala-Aca-Bala spacer arm film plate as used in the preferred embodiment for ASBCL or ASPCL libraries.

Example 5 - Construction of A 10x10 Hexapeptide ASPCL Plate:

The method of this invention allows the construction of arrays of sequences at any 2 sequence positions within peptides of any reasonable size with several positions being incorporated as mixtures. The preferred embodiment of the method is to prepare hexapeptides with the central 2 AA's arrayed, the other 4 positions redundantly mixed, and the final sequence AA is N-acetylated. For film plates it is preferred to add a spacer arm peptide to the film prior to construction of the library, and with beaded plates an octapeptide spacer is attached prior to embedding in the glue. The following sequence of operations is followed to prepare a Hexapeptide ASPCL with a known central (3,4) AA sequence:

i) Apply an edge gasket and a border-frame spacer on the substrate plate (see Fig. 5) to make a flat "dish" type reactor. As an alternative to use of the screwed pin base plate assembly of Fig. 1, one may use standard large office clips, or wing nuts and standard bolts and washers to hold the parts together;

ii) Wash with DMF 2x using horizontal action shaker;

iii) Couple 10 Ml preactivated MIX solution made as per Example 1 for 2 hours, while covering plate with foil tent;

iv) Wash with DMF 3x;

v) Deblock with 30% piperidine in DMF 1 min, 10 min;

vi) Wash with DMF 5x;

vii) Repeat steps iii) to vi);

viii) Dismount edge gasket and frame spacer, and mount slotted block assembly with 100 hole gasket to base plate as in Fig. 1 with slots in a first, horizontal orientation (L TO R when facing the assembly). DMF solvent is placed in alternate wells and the dry wells observed carefully to ensure no leakage. Prepare in vials the following amino acids: 1. Fmoc-L-Nle-OH 0.14g; 2. Fmoc-L-His(Trt)-OH 0.25g; 3. Fmoc-L-Pro-OH 0.135g; 4. Fmoc-L-Gln(Trt)-OH 0.244g; 5. Fmoc-Tyr(Tbu)-OH 0.183g, 6. Fmoc-Gly-OH 0.116g; 7. Fmoc-L-Phe-OH 0.154g; 8. Fmoc-L-Arg(Pmc)-OH 0.265g; 9. Fmoc-L-Glu(OtBu)-OH 0.17g; 10. Fmoc-D-Ala-OH 0.124g. To each of these vials add and mix PyBOP 0.27g and HOBt 0.06g, and 2 Ml of 0.3M N-methylmorpholine in DMF. Add each to a designated horizontal slot: 1 to top slot; 2 to next slot, etc.;

ix) Maintain at room temperature for 2 hours to complete coupling;

x) Disassemble and remount with edge-gasket and border-frame spacer;

xi) Wash with DMF 3x;

xii) Deprotect with 30% piperidine in DMF 1 minute, 10 minutes;

xiii) Wash with DMF 5x;

xiv) Mount slot block with slots rotated 90°, i.e., in a vertical orientation, and repeat coupling as described in viii) except 1 is coupled to the left hand slot, 2 to the next slot, etc.;

xv) Disassemble and remount with edge-gasket and border-frame spacer;

xvi) Remove Fmoc group and perform 2 cycles of mixture incorporation as described in i) to vii);

xvii) Remove Fmoc-group and wash with DMF 5x;

xviii) Acetylate with 0.3M acetic anhydride + 0.3M HOBt in DMF (10 Ml) for 1 hour;

xix) Wash DMF 5x, Methanol 5x;

xx) Treat with 95:5 TFA/water for 2 hours;

xxi) Wash Methanol 5x, aqueous buffer 5x. Store in sealed bag at 4 degrees prior to probing to screen a target.

Numbering from the carboxy terminus, the resulting hexapeptides are characterized as XX-A_4, A_3-XX with the A_3 and A_4 known sequence being uniquely addressable. That is, the central dipeptide is known from its unique address by use of the slotted block, the hexapeptide at address 001 being XX-D-Ala-L-Nle-XX, at address 002 being XX-L-Glu-L-Nle-XX, etc., to address 100 being XX-L-Nle-D-Ala-XX.

Example 6 - Construction of A 10x10 Hexapeptide ASPCL on a Gel Film Plate

Instead of a bead plate, a gel film plate as in Examples 3 and 4 may be similarly employed to construct an ASPCL library by the process of Example 5. This gel film ASPCL is used to screen, see Example 7 below.

Example 7 - Determination of Binding Elements in the Interaction of Streptavidin with Peptides

A film library plate of Examples 3 and 4 was constructed similarly to Example 6 with the selection of 10 amino acids as indicated. ^{125}I labelled streptavidin was prepared and purified by standard procedures; a fluorescently labelled form was also prepared by reaction of AMCA-NHS (Pierce) with the protein, excess reagent being removed by dialysis. Firstly, the iodinated protein was incubated with the plate overnight in a phosphate buffer containing 150 Mm salt, Tween detergent and bovine

serum albumin (1mg/Ml). The plate was washed with the buffer 3 times, placed on a standard laboratory X-ray film with an enhancer plate and exposed overnight. The developed film shows strong affinity in specific address locations corresponding to 2 central dipeptides HP and RR. The plate was then washed repeatedly with 6M guanidine hydrochloride, and buffer medium, then reincubated with the fluorescent AMCA-streptavidin overnight. After washing the plate was irradiated with long wave length uv light and visible confirmation obtained of the previously deduced binding elements. Subsequent iterations as described above further defines the active dipeptides at each end for complete hexapeptide characterization.

Example 8 - Array Test for ^{35}S Streptavidin Using HPMP Winks in a Carrier Plate

This example details the use of the HPMP winks in the carrier plate 5' of Fig. 8a with the slotted block system of Fig. 6 and vacuum base system of Fig. 7.

70 dextran functionalized winks were placed in the reaction vessel of a Millipore Model 9600 peptide synthesizer. A mixture of Fmoc-protected amino acids was made by carefully weighing the individual components according to the following list, followed by intimately mixing them in a pestle and mortar: L-NorLeu, 0.093g; L-His(Trt), 0.341 g; L-Pro 0.10g; L-Gln(Trt), 0.351g; L-Tyr(Tbu), 0.203g; Gly, 0.046g; L-Phe, 0.102g; L-Arg(Pmc), 0.52g; L-Glu(OtBu), 0.132g; L-Ala, 0.064g; D-Ala, 0.064g; L-Asp(OtBu), 0.150g; L-Val, 0.131g; L-Ser(Tbu), 0.149g; D-(2-napthyl)Ala, 0.153g; L-Lys(Tboc), 0.168g. Four individual aliquots of the mixture (0.461g) were placed along with PyBOP (0.82g) and HOBt(0.2g) in each of the first 4 reservoirs of the instrument, and synthesis performed using standard Fmoc 4 hour coupling programs with 10 minute preactivation.

Following synthesis, 64 of the product winks, now bearing tetrapeptide mixtures of all possible combinations, were placed in an 8x8 array in the standard 10x10 plate with blank winks occupying peripheral

positions. The plate was marked in its top right hand corner, washed with DMF several times, then placed in the slotted block system of Fig. 6 with a solid viton gasket between it and the base plate. The standard 100 hole top gasket was then positioned, followed by the slotted block in a vertical orientation. The slots were tested to make sure no leakage was occurring. For array coupling, pairs of amino acids were added to the 8 appropriate slots as follows (each pair also had added 0.80g PyBOP and 0.2 g HOBt, activation was with 5 Ml of 0.3M NMM in DMF): - Slot 2, Tyr(Tbu) 0.29g + Phe 0.143g; Slot 3, Asp(OtBu) 0.22g + Glu(OtBu) 0.195g; Slot 4, Arg(Pmc) 0.457g + Lys(Tboc) 0.145g; Slot 5, Nle 0.134g + Val 0.21g, Slot 6, Gly 0.128g + D-Ala 0.177g; Slot 7, Ala 0.109g + Ser(Tbu) 0.249g; Slot 8, His(Trt) 0.29g + Gln(Trt) 0.312g; Slot 9, Pro 0.146g + DNapAla 0.25g. The apparatus was left to gently shake overnight to insure coupling.

The apparatus was disassembled, the plate 5′ placed in the vacuum block assembly of Fig. 7, washed with DMF (6x), deblocked with 30% piperidine in DMF for 10 minutes, washed DMF (6x). Plate 5′ was placed back in the slotted block system of Fig. 6 with the slotted block being turned 90°. An identical array coupling was performed except that the slots were now oriented in a horizontal manner. After a 4 hour coupling, the plate 5′ was transferred back to the vacuum base apparatus of Fig. 7, washed with DMF (6x), deblocked with 30% piperidine in DMF (10 minutes), then washed with DMF (6x) and methanol (6x). The plate 5′ carrying the winks was then treated to remove all side-chain protecting groups for 4 hours with 90% TFA, 5% anisole, 2.5% water, 2.5% dimethyl sulfide. Subsequently it was washed 6 times with methanol, then DMF, water, methanol and water. It was then equilibrated overnight in the assay buffer consisting of phosphate buffered saline (PBS) containing 0.2% Tween 20 detergent and 1 mg/Ml of bovine serum albumin.

To probe the plate, fresh buffer was added (20 Ml), and 100 microliters of standard Amersham ^{35}S labeled streptavidin solution added. The probing was rocked

gently for 2 hours, then the supernatant sucked out via the vacuum base. The plate was then washed rapidly whilst rocking with 2 x 20 Ml of assay buffer, then 2x 20 Ml of water. The plate was then separated from the apparatus, inverted, and individual winks poked (Fig. 8a) out into clearly labeled corresponding scintillation vials containing 0.5 Ml of 0.1M Hcl. These vials were shaken for 1 hour to displace bound activity. Standard scitillation cocktail (10 Ml) was added, then each vial counted for 5 minutes on a Beckman beta-counter.

Results according to array location are depicted in Fig. 11. Surprisingly, many areas have absorbed radioactivity. Four main peaks were selected, and the 16 possible dipeptides (GF, GY, DAY, dAF, NleG, NleDAla, VG, VdA, NleY, NleF, VY, VF, NleNle, NleV, VNle, VV were produced on $(Mix)_4$ winks with the aid of a modified multiple peptide synthesizer. Following side-cahin deprotection and probing, VY and VF showed maximal binding with >90% of absorbed counts being displaced with biotin.

A second array series was then performed. $(Mix)_2$ winks were made and mounted in the plate. The array steps were then performed in the central 2 positions of the hexapeptide. First Y, then V were added to complete the process. Deprotection, probing and synthesis of possible selections found VYGF and VYHP as strong binders.

A third array series was then performed with the C-terminal 2 positions arrayed and VYGF appended thereto. Probing showed VYGFRQ as the best combination. Following up a VYHPQ lead, we found VYHPQF and VYHPQV to be good binders, slightly better than HPQFVbA, and our own best sequence HPQVFV. To test whether the two series of peptides overlapped at the biotin binding site of streptavidin, a combination nona-peptide, HPQVYGFRQ, was made and found to be a much stronger binder by both BIAcore and PILOT comparison.

This example illustrates the true potential of the PILOT method for drug discovery. Its high sensitivity, utilizing arrays prepared by the simple system of this invention with optimal display chemistry of the HPMP

winks, allows the identification of weak binding elements, which when combined and properly oriented permit advanced pattern recognition for mapping of receiptors to yield new highly active drug candidates and high affinity super-binding compounds.

It should be understood that various modifications within the scope of this invention can be made by one of ordinary skill in the art without departing from the spirit thereof. We therefore wish our invention to be defined by the scope of the appended claims in view of the specification as broadly as the prior art will permit.

CLAIMS

We Claim:

1. A biological screening assembly comprising in operative combination:

a) an inert support plate having defined thereon an array of discreet, individually addressable areas for receiving a biopolymer-retaining substrate material;

b) at least one biopolymer-retaining substrate material secured to each of said addressable areas; and

c) said substrate material on at least some of said areas of said array is selected from the group consisting essentially of a polymer disc, a polymer film, aparticulate material, and combinations thereof, and said substrate material is amino-functionalized for assembly of biopolymer chains thereon.

2. A biological screening assembly as in claim 1 wherein:

a) said polymer film on at least some of said areas of said array is selected from the group consisting essentially of a film polymerized in situ, at least partly preformed polymer, and combinations thereof;

b) said plate areas include active groups for chemical bonding with components forming said films; and

c) said substrate includes at least one functionalized spacer arm derivative in at least some of said areas.

3. A biological screening assembly as in claims 1 or 2 wherein:

a) said plate is a polyolefin;

b) said film is a polymer having low molar percentage of crosslinking covalently grafted to said active groups in said areas to form said biopolymer-retaining substrate; and

c) said spacer arm is amino-functionalized.

4. A biological screening system comprising in

operative combination:

a) an inert support plate having defined thereon an array of discreet, individually addressable areas for receiving a biopolymer-retaining substrate material;

b) at least one biopolymer-retaining substrate material secured to each of said addressable areas.

c) a barrier gasket sheet overlying said support plate having an array of holes therethrough aligned with said array of areas for access to said substrate areas; and

d) an apertured block disposed on said gasket having a plurality of slots each oriented to provide a common well for simultaneous access to a plurality of said substrate areas in said array.

5. A biological screening system as in claim 4 wherein:

a) said array is selected from a rectangular N x N aray wherein N rages from 2 to 400;

b) said slotted block is selected from a corresponding block having N linear slots positioned to provide access to all the substrate areas of said plate; and

c) said block is orientable on said array in at least two rotationally different positions with respect to each other to permit construction of defined sequences from chemical components placed in said wells.

6. A biological screening system as in claims 4 or 5 wherein:

a) said block includes means for identifying the orientation of said slots to said substrate array to provide accurate orientation upon each rotation.

7. A biological screening assembly as in any one of claims 1-6 wherein:

a) at least some of said addressable areas comprise porous polyolefin discs removably received in

holes in said plate; and

b) said substrate material on said discs is a hydrophilic polar multi-functionalized polymer film.

8. A biological screening system comprising in operative combination:

a) an inert support plate having defined thereon on array of discreet, individually addressable areas for receiving a biopolymer-retaining substrate material;

b) at least one biopolymer-retaining substrate material secured to each of said addressable areas;

c) at least some of said addressable areas comprise porous polyolefin-discs removably received in holes in said plate, and said substrate material on said discs is a hydrophilic polar multi-functionalized polymer film;

d) a border block overlying said support plate;

e) a vacuum base underlying said plate; and

f) means to seal said plate between said border block and vacuum plate to permit withdrawal of solution applied on said plate through said porous disc and said plate holes.

9. A method of preparing chemical polymer sequences on a continuous support substrate comprising the steps of:

a) superimposing on a substrate a first block having a first top and a first bottom and a pulurality of first slots disposed to extend vertically between said first top and bottom;

b) adding a first pulrality of solution of protected monomers to each of the first slots, wherein the solution in each slot comprises a different known protected monomer;

c) reacting the protected monomer solutions with the substrate to attach the protected monomer to the substrate in at least selected areas within the slot and form reacted substrate areas;

d) deprotecting at least some of the reacted substrate areas;

e) superimposing on the reacted substrate areas a second block having a second top and a second bottom and a plurality of second slots disposed to extend vertically between said second top and bottom, said second block being oriented with its plurality of second slots forming an orthogonal set with said plurality of first slots;

f) adding a second plurality of solutions of protected monomers to each of the second slots, wherein the solution in each slot comprises a different known protected monomer; and

g) reacting the second protected monomer solutions with the reacted substrate areas to attach the second protected monomer to the reacted substrate areas to form an array of polymer sequences having known monomers in known positions in said sequences at known address locations in said array.

10. The method of claim 9 wherein:

a) said plurality of first slots is a plurality of linear parallel slots;

b) said plurality of second slots is a plurality of linear parallel slots; and

c) step (e) is performed by rotating said first block 90°.

11. The method of claims 9 or 10 wherein:

a) other moieties may be interposed and reacted with the deprotected substrate of step (d) before reacting the second plurality of solutions of steps (e), (f) and (g)';

b) the monomers are selected from the group consisting essentially of protected L-, D- and non-natural amino acids, protected DNA monomers, protected RNA monomers, protected monosaccharide units, and mixtures thereof;

c) said first and said second protected known

monomers are attached in said polymer sequence in any predetermined order selected from sequential to each other to separated by other moieties.

12. The method of any of claims 9-11 wherein:

a) the positions in said polymer sequence other than occupied by said known monomers are selected from the group consisting essentially of random equimolar monomers and polymers; and

b) the monomers are selected from the group consisting essentially of protected L-, D- and non-natural amino acids, protected DNA monomers, protected RNA monomers, protected monosaccharide units, and mixtures thereof.

13. The method of any of claims 9-12 wherein:

a) the positions in said polymer sequence other than occupied by said known amino acids are selected from the group consisting essentially of random equimolar mixed amino acids, dipeptides, tripeptides and polypeptides.

14. The method of any of claims 9-13 which includes the added steps of:

a) screening said array of polymer sequences having two known monomers in any known position against a preselected target to locate by address in said array on said substrate at least one polymer sequence having a desired property;

b) determining the known monomer sequence by reference to the address in said array on said substrate;

c) repeating the steps of reacting protected known monomers at determined positions in said polymer sequence different than the positions of said first and second known monomers to form a second array;

d) repeating said screening against said preselected target;

e) repeating said determination of the known monomers sequence; and

f) repeating steps c, d and e at least one additional time to fully characterize the polymer sequence active with respect to said target.

15. The method of any of claims 9-14 wherein:

a) said monomers are L-, D- and non-natural amino acids; and

b) said polymer sequence is a hexapeptide forming an ASPCL.

16. A method of diagnosis for biologically active target molecules comprising the steps of:

a) providing an ASBCL plate having an addressable array of known target active sequences thereon;

b) exposing said ASBCL plate to at least one solution expected to contain at least one target; and

c) identifying any target in said solution by binding at an address on said array that is site specific for said target.

17. A method of diagnosis as in claim 16 wherein:

a) said ASBCL plate array includes at least some peptide sequences thereon.

18. A method of diagnosis as in claims 16 or 17 wherein:

a) said ASBCL is an ASPCL.

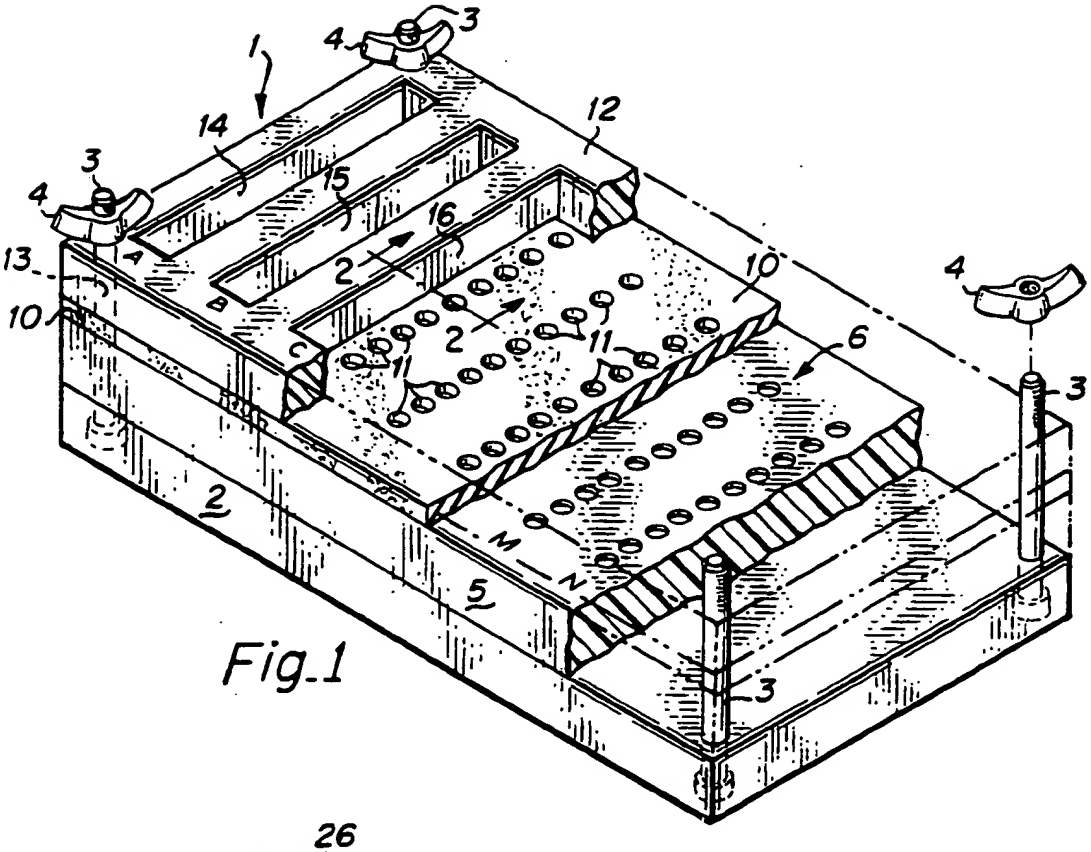
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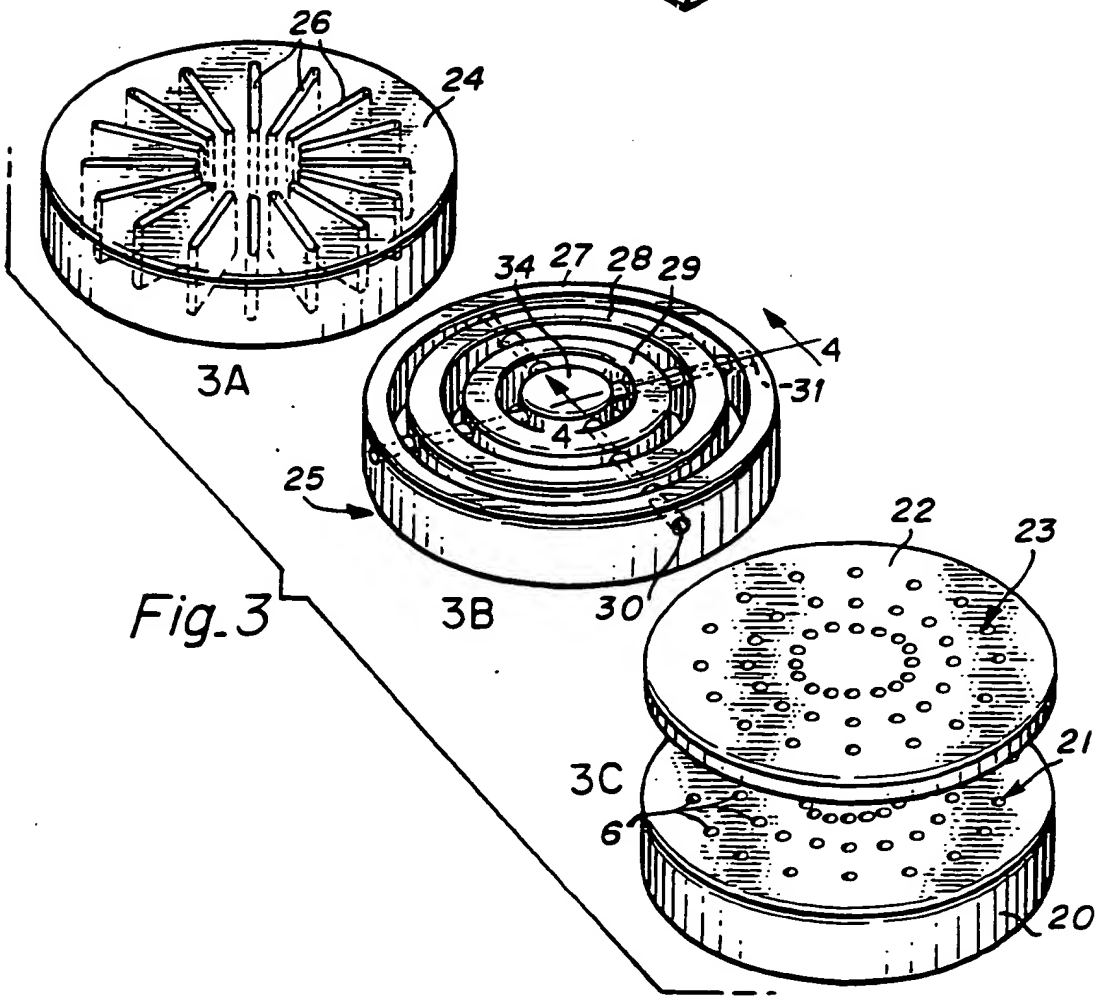
Fig_1


3A
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3C
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Fig_3

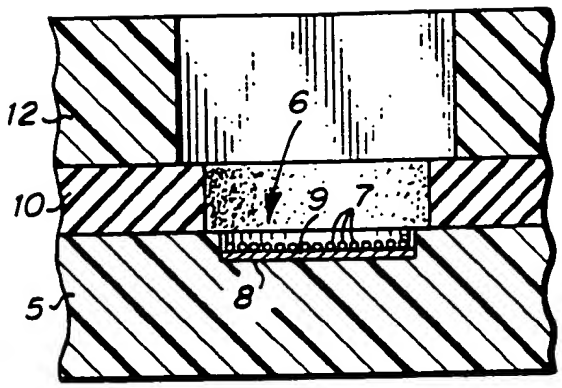


Fig_2a

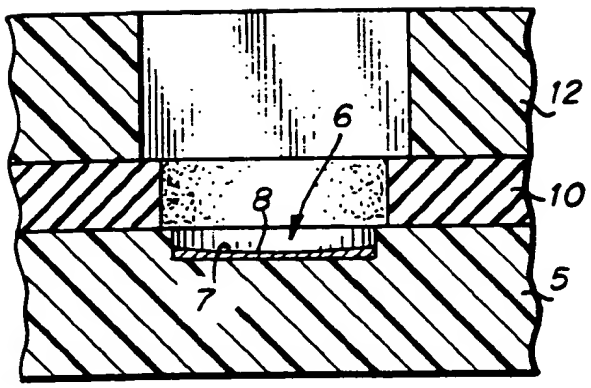


Fig_2b

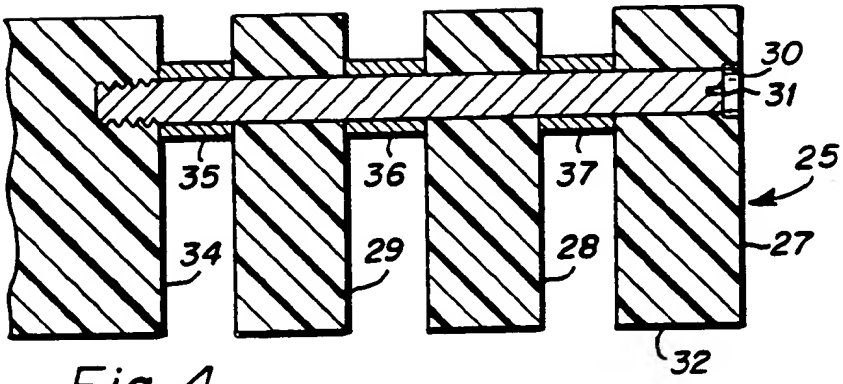


Fig_4

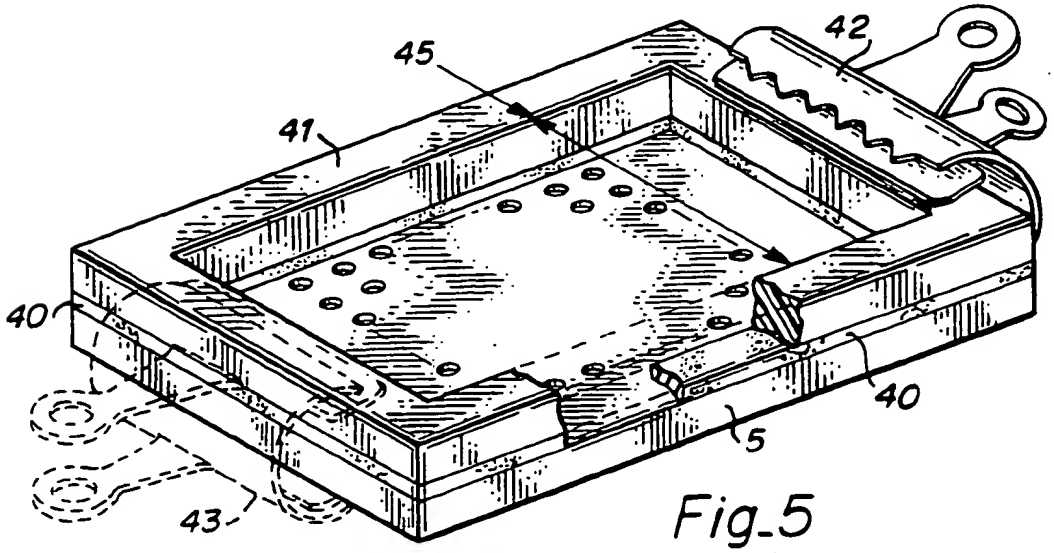


Fig_5

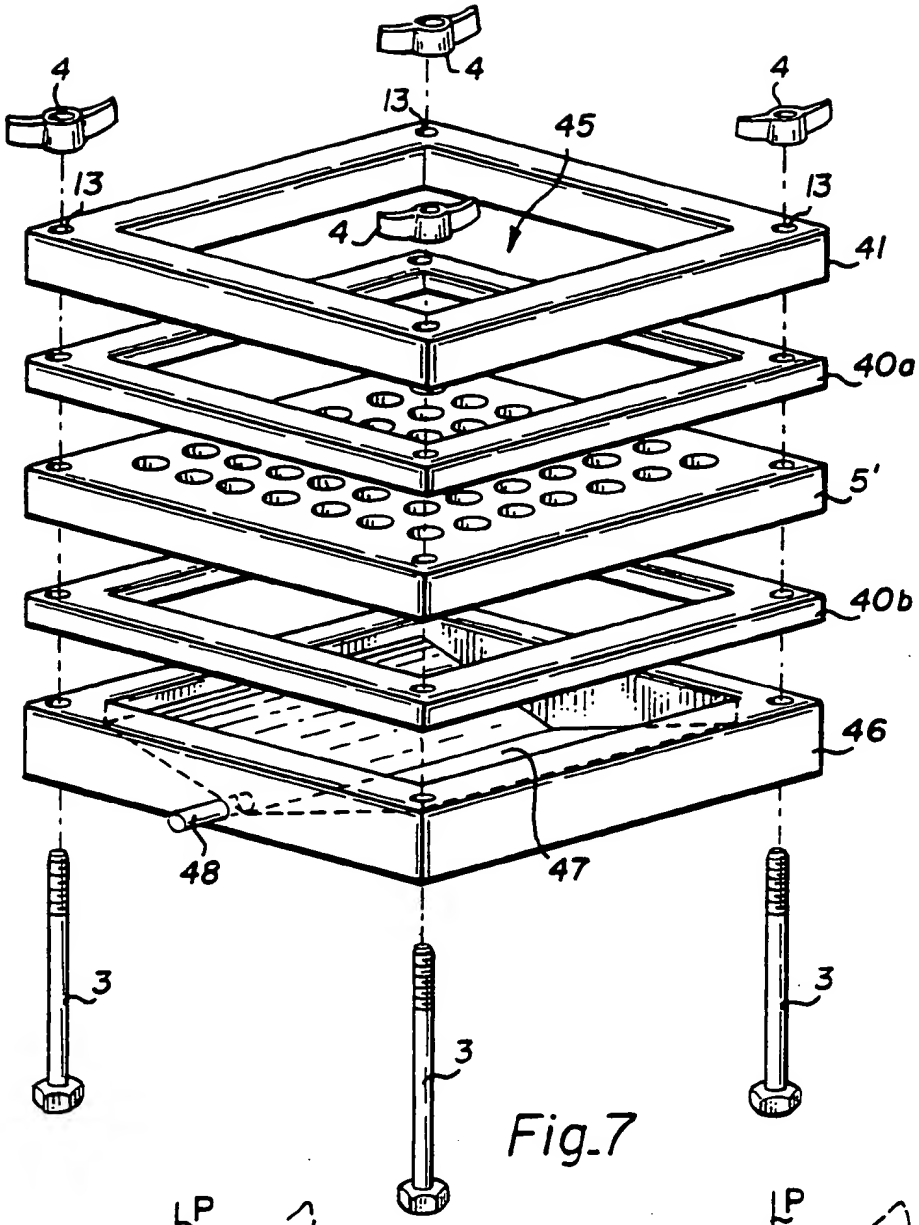


Fig_7

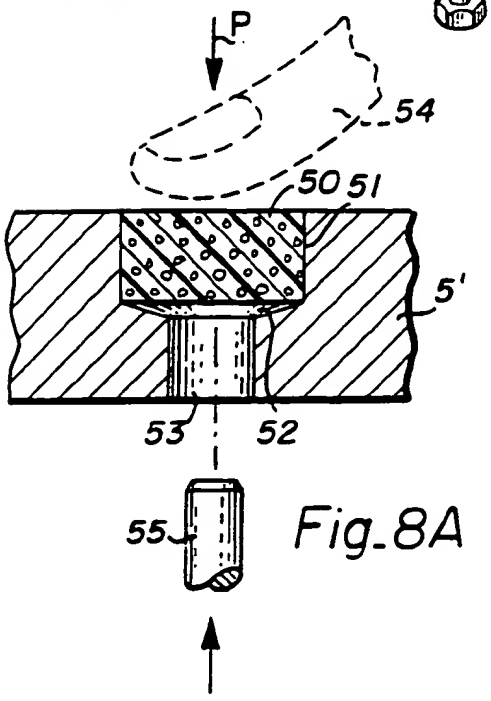


Fig_8A

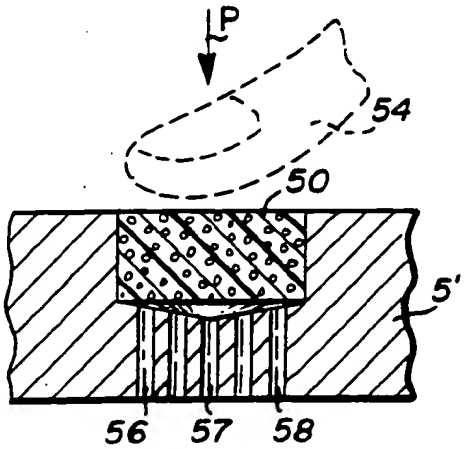


Fig_8B

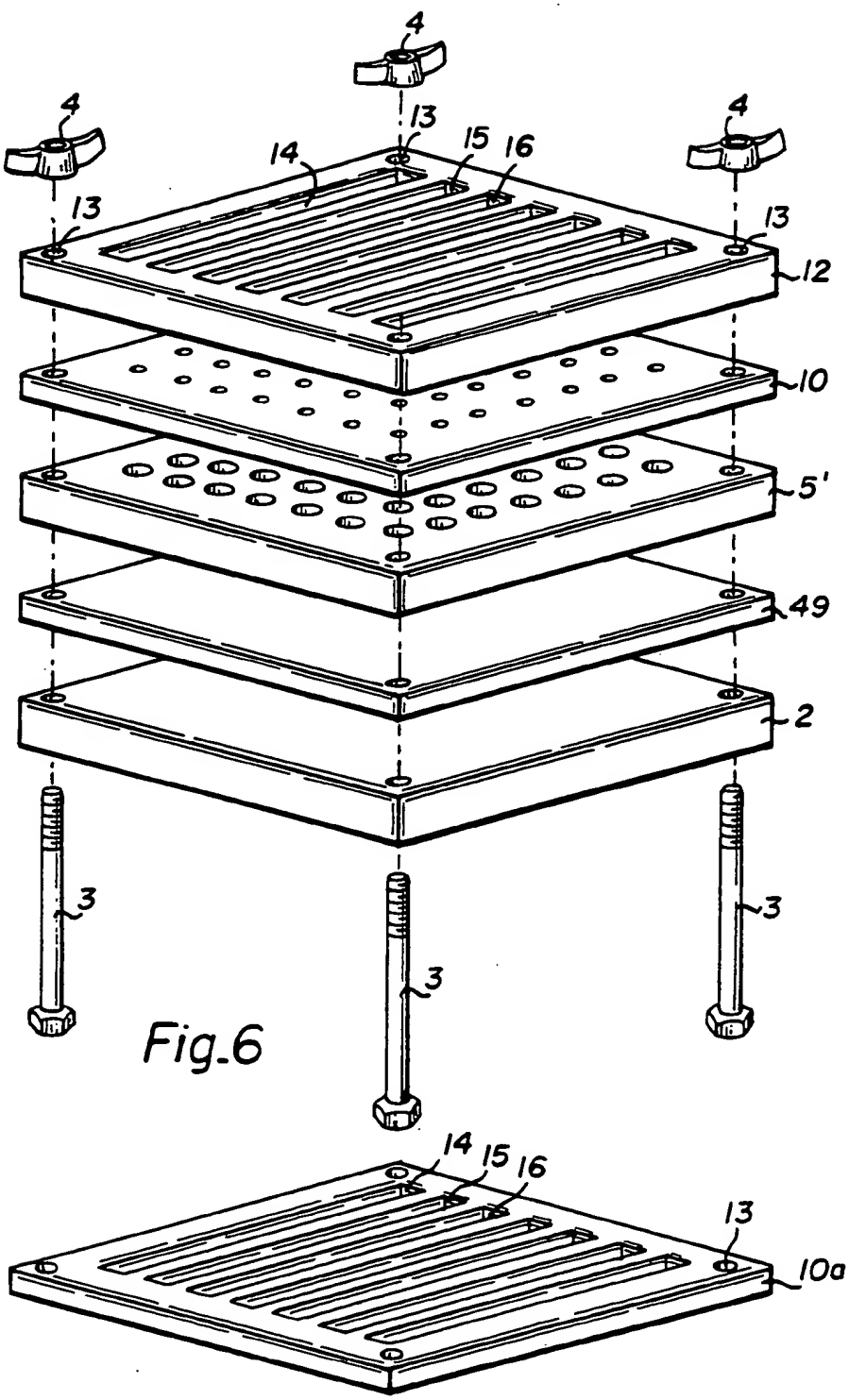
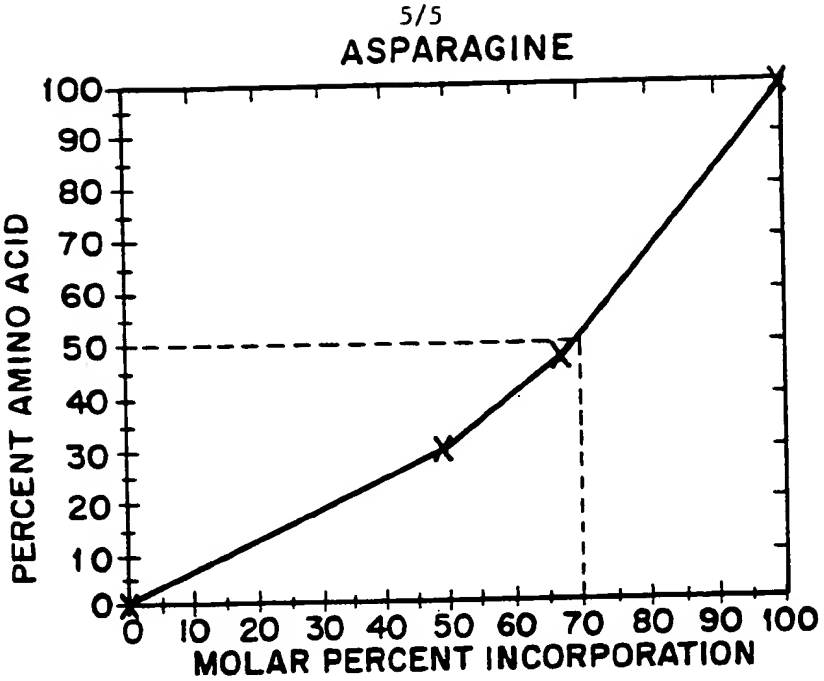


Fig_6

Fig_9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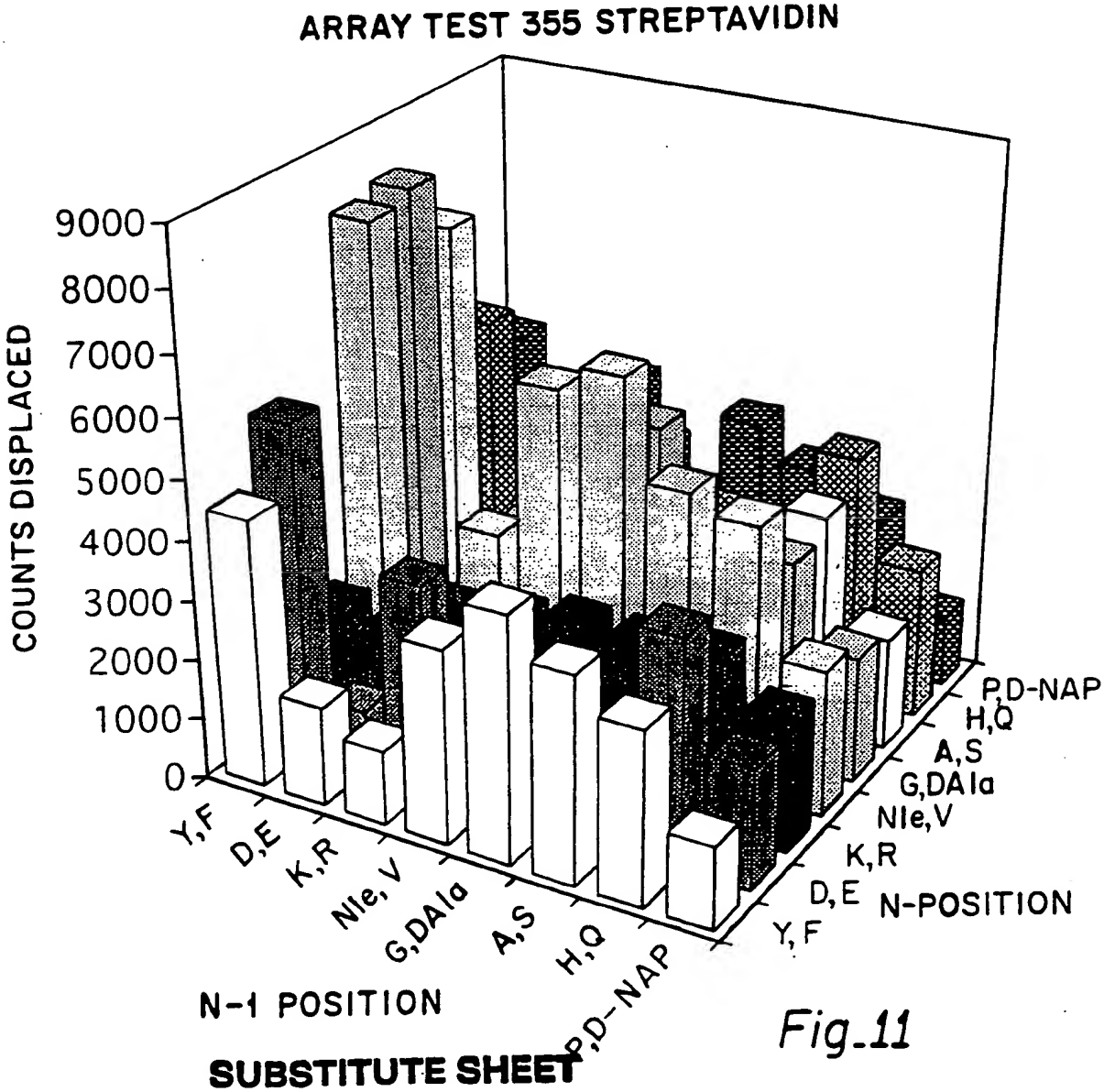
MOLAR PERCENT INCORPORATION

Fig_10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5000
4000
3000
2000
1000
0
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D,E
K,R
Nle,V
G,DAla
A,S
H,Q
P,D-NAP
N-1 POSITION
N-POSITION

Fig_11

INTERNATIONAL SEARCH REPORT

Intern. .onal application No.
PCT/US93/08267

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,728,502 (Hamill) 01 March 1988, abstract, Fig. 4, col. 1, lines 49-66, col. 3, lines 13-26 and 58-62.	1-5, 8-11
X Y	US, A, 5,100,626 (Levin) 31 March 1992, abstract, Figs. 2-3, col. 3, lines 37-50, col. 4, lines 20-25 and 46-55, col. 5, line 18 - col. 6, line 11, col. 7, lines 25-40, col. 8, lines 35-42, col. 9, lines 8-20	16-18 1, 4, 6, 8-9, 11, 14-15
X Y	WO, A, 90/02605 (Meldal et al) 22 March 1990, abstract, page 1, lines 3-9, page 3, lines 13-16, page 6, line 24 - page 7, line 27, page 12, line 29 - page 15, line 29, Figs. 3, 6-8 and 16.	1 2-3, 9-13

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 01 DECEMBER 1993	Date of mailing of the international search report 16 DEC 1993
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer CAROL A. SPIEGEL Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

Intern...onal application No.
PCT/US93/08267

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	Genomics, volume 13, issued August 1992, E. Southern et al, "Analyzing and Comparing Nucleic Acid Sequences by Hybridization to Arrays of Oligonucleotides: Evaluation Using Experimental Models", pages 1008-1017, especially abstract, pages 1008-1011.	16-18 1-2, 4-5, 8-9, 11-15
X Y	US, A, 5,143,854 (Pirrung et al) 01 September 1992, abstract, Figs. 10A-10M, col. 3, line 9 - col. 4, line 27, col. 7, lines 49-62, col. 8, lines 1-7, col. 11, lines 31-38 and 51-68, col. 12, lines 43-45, col. 15, lines 9-22, col. 16, lines 18-21, 28-32 and 46-52, col. 17, line 66 - col. 18, line 3, col. 18, lines 40-68, col. 19, lines 34-37, col. 20, lnes 10-32, col. 23, lines 19-67, col. 24, lines 12-14.	1, 16-18 2-15
Y,P	US, A, 5,188,733 (Wang et al) 23 February 1993, Figs. 1 and 3-6, col. 2, line 38 - col. 3, line 3, col. 3, lines 25-33 and 43-61, col. 4, lines 17-19, 49-51 and 56-57, claims 1-8	1-4, 7, 9, 11, 14
Y	Journal of the American Chemical Society, vol. 111, no. 20, issued 27 September 1989, R. Berg et al, "Long-Chain Polystyrene-Grafted Polyethylene Film Matrix: A New Support for Solid-Phase Peptide Synthesis", pages 8024-8026, especially pages 8024-8025.	1-4
Y	Tetrahedron Letters, vol. 33, no. 21, issued 19 May 1992, M. Meldal et al, "PEGA: A Flow Stable Polyethylene Glycol Dimethyl Acrylamide Copolymer for Solid Phase Synthesis", pages 3077-3080, especially pages 3077-3078.	3
Y,P	Tetrahedron Letters, vol. 34, no. 10, issued 05 March 1993, S. Kates, "A Novel, Convenient, Three-Dimensionsal Orthogonal Strategy for Solid-Phase Synthesis of Cyclic Peptides", pages 1549-1552, especially page 1549.	9
A	US, A, 5,053,454 (Judd) 01 October 1991, see entire document.	1-18
A,P	US, A, 5,147,608 (Hudson et al) 15 September 1992, see entire document.	1-9
A,P	US, A, 5,175,209 (Beattie et al) 29 December 1992, see entire document.	1-18
A,P	US, A, 5,196,566 (Barany et al) 23 March 1993, see entire document.	1-18

INTERNATIONAL SEARCH REPORT

Inter..onal application No.
PCT/US93/08267

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	US, A, 5,258,454 (Berg et al) 02 November 1993, see entire document	1-8

INTERNATIONAL SEARCH REPORT

Intern...onal application No.
PCT/US93/08267

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (5):

B01D 25/12, 63/00; B01J 19/00; B01L 9/00, 11/00; C08F 283/00, 285/00; C07H 21/00; C07K 1/06, 5/00, 7/00, 15/00, 17,00; C08L 89/00; C12Q 1/00; C12P 19/04, 19/38, 21/06; G01N 21/00, 33/00, 33/543, 33/544, 33/545, 33/546, 33/552

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

210/321.84, 321.75, 323.1, 450, 638; 422/ 56, 57, 58, 101, 102, 103, 104, 130, 131, 134, 136, 191, 209; 427/2, 491; 435/7.1, 7.92, 7.94, 69.1, 188, 287, 293, 301, 312; 436/89, 94, 518, 527, 528, 529, 531, 532; 525/54.1, 54.11; 530/333, 334, 335, 336, 337

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

210/321.84, 321.75, 323.1, 450, 638; 422/ 56, 57, 58, 101, 102, 103, 104, 130, 131, 134, 136, 191, 209; 427/2, 491; 435/7.1, 7.92, 7.94, 69.1, 188, 287, 293, 301, 312, 961, 973; 436/89, 94, 518, 527, 528, 529, 531, 532, 807; 525/54.1, 54.11; 530/333, 334, 335, 336, 337 935, 79, 80, 81, 82, 87, 88

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, DIALOG
search terms: solid phase synthesis or preparation, peptide, polypeptide, oligonucleotide, polysaccharide, amino or amine function? or derivati?, spacer, linker, handle, channel or slot block, gasket, seal, vacuum, polyolefin, polyethylene, dextran, polyethylene glycol, chitin, chitosan, polydimethylacrylamide, silica, polystyrene, crosslink?, kieselguhr

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

I. Claims 1-3 and 7, drawn to an apparatus comprising a solid phase polymeric support having a substrate matrix of individually defined amino-functionalized areas for chemically anchoring biopolymers thereto, wherein the substrate is preferrably a polymer film having low molar percentage of crosslinking covalently grafted to the support, classified in class 435, subclass 174..

II. Claims 4-7, drawn to a channel block apparatus comprising a common well access to an inert support having a substrate matrix of individually defined areas for attaching biopolymers thereto, wherein the substrate preferrably comprises a hydrophilic polar multi-functionalized polymer film attached to a support preferrably comprising porous polyolefin discs, classified in class 422, subclass 131.

III. Claim 8, drawn to an apparatus for synthesizing biopolymers comprising a support having individual holes containing a hydrophilic polar multi-functionalized polymer film attached porous polyolefin discs, sandwiched between an overlying border block and an underlying vacuum base, and a sealing means capable of providing fluid flow through the porous discs, classified in class 422, subclass 104.

IV. Claims 9-13, drawn to methods for synthesizing polymers in an apparatus comprising a plurality of rotatable slot blocks and using random equimolar monomers and polymers comprising protected amino acids, protected nucleic acids and protected monosaccharides, classified in class 530, subclass 333.

V. Claim 14, drawn to a method of synthesizing and individually screening an array of discrete polymer sequences on a substrate for reactivity against a preselected target, classified in class 436, subclass 518.

VI. Claims 15-18, drawn to methods for screening a synthetic peptide combinatorial library in order to determine which polypeptides specifically bind to a known target receptor, classified in class 435, subclass 7.1.